Flora xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

Leaf traits of C_3- and C_4-plants indicating climatic adaptation along a latitudinal gradient in Southern Siberia and Mongolia☆

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ARTICLE INFO

Edited by Hermann Heilmeier

Keywords:
Plant functional traits
Leaf morphology
Quantitative leaf anatomy
Mesophyll surface
Photosynthetic performance
Desert steppe
Aridity gradient

ABSTRACT

Increasing aridity is one of the most important trends of current climate change. Leaf functional traits suggest a substantial basis for assessing the aridity effects on vegetation. However, since plants possess diverse leaf morphology and anatomy due to different evolutionary history of taxa, the effect of aridity can hardly be revealed in a multi-species analysis. We studied leaf functional traits for 317 samples of 193 plant species in steppe and desert communities along a 1600-km latitudinal gradient in Southern Siberia (Transbaikalia, Russia) and Mongolia. We determined morphological leaf traits, quantitative anatomical parameters, physiological parameters, and photosynthetic pigments content. Different relevance of leaf traits for indication of plant response to climate has been demonstrated. The clearest changes in site-mean values along the aridity gradient were shown for leaf thickness, total chloroplast number per leaf area (N_{chl}/A) and total surface area of chloroplasts (A_{chl}/A) and cells (A_{mes}/A) per leaf area. Unlike leaf size and leaf mass per area, these quantitative mesophyll parameters related to plant photosynthetic capacity were strongly correlated with climate. We found no evidence for a decrease in sizes of mesophyll cells with aridity, but cell volume as well as chloroplast number per cell were linked with plant functional type (PFT). We revealed an increase in N_{chl}/A and A_{chl}/A in desert-steppe species in comparison to steppe and forest-steppe vegetation types within each PFT of C_3-plants (C_3-dicot herbs, C_3-dicot shrubs, C_3-monocots and C_3-succulents). C_4-plants were generally characterized by low A_{chl}/A and A_{mes}/A, but had higher rate of CO_2-transfer through mesophyll and chloroplast surfaces. C_3- and C_4-plants differed in response to aridity and showed opposite trends in changes of leaf traits along the aridity gradient. We conclude that leaf mesophyll traits contribute to important mechanism of climatic adaptation in different PFTs along a large latitudinal gradient.

Nomenclature

Catalogue of Life (www.catalogueoflife.org; accessed on 1 February 2018), and The Plant List (http://www.theplantlist.org; accessed on 1 February 2018).

1. Introduction

Increasing aridity is one of the most important climatic factors of current climate change in step with a general global trend towards climate warming and drying (Burke et al., 2006; Hansen et al., 2010;

☆ This article is part of a special issue entitled: "Functional traits explaining plant responses to past and future climate changes" published at the journal Flora 254C, 2019.

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https://doi.org/10.1016/j.flora.2018.10.008
Received 12 March 2018; Received in revised form 12 September 2018; Accepted 12 October 2018
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Cook et al., 2014). Aridification is a complex climatic process combining long-term changes in temperature, the amount and seasonality of precipitation and insolation. These environmental variables are among the key determinants of physiology, biomass allocation and fitness of organisms, and increasing aridity is thus expected to have profound consequences on biodiversity and the distribution of vegetation types (Woodward, 1987; Chapin et al., 2000; Parmesan, 2006; Rustad, 2008). Studying plant responses to climate across latitudinal gradients is a useful approach to examining the effects of climate warming and aridity on vegetation (Reich and Oleksyn, 2004; De Frenne et al., 2013). Vegetation of current sites has evolved with the local climate over centuries (Reich et al., 2003; Rustad, 2008; Castro-Diez, 2012). Therefore a study along a latitudinal gradient provides excellent opportunities to examine the long-term effects of increasing temperature and dryness on plant communities (De Frenne et al., 2013).

Leaf functional traits suggest a substantial basis for assessing the effects of aridity on vegetation (Voronin et al., 2003; Poorter et al., 2009; Gillison, 2013). That plants from drier sites possess thicker and denser leaves than plants from humid areas is probably one of the earliest observations in plant ecology (Schimper, 1898; Maximov, 1929). Leaf mass per unit area (LMA) is often referred as one of the indices of sclerophylly (Witkowski and Lamont, 1991; Turner, 1994; Fonseca et al., 2000) which is interpreted as an adaptation to water deficit (Shields, 1950). However, sclerophylly is also known to be a defense against herbivory (Turner, 1994) and as an adaptation to a nutrient deficit (Beadle, 1966). High LMA can be found not only in dry sites, but in some species in wetlands (Ronzhina and Pyankov, 2001). Significant differences in values of LMA as well as of leaf thickness (LT) may be clearly revealed in contrasting environments, between sites with high and low water availability (Mokronosov, 1981; Poorter et al., 2009; Ivanova, 2014; de la Riva et al., 2016). The use of whole-leaf traits along detailed geographical gradients where only small shifts in aridity between nearby sites are noted, does not often allow us to identify a clear effect of aridity (Ivanov et al., 2009). Plant species that inhabit the same site may possess a different leaf morphology and anatomy due to the different evolutionary history of taxa. Although having similar values of LMA and leaf thickness (LT) plant species may differ in leaf internal structure and physiology. Diverse anatomical / morphological and physiological adaptations to aridity have originated across many plant groups (Gamalei, 1988; Chaves et al., 2002; Galmes et al., 2012). Therefore it seems unlikely that the effect of aridity can be revealed in a multi-species analysis.

Photosynthesis is among the primary processes to be affected by temperature and drought stress and among the critical constraints to primary productivity in arid conditions (Flexas et al., 2004; Chaves et al., 2009; Galmes et al., 2012). Physiological adaptation to aridity at the leaf level necessarily concerns photosynthetic functioning. Apparent evidence for this is the climatic distribution of metabolic types of photosynthesis. Earlier, direct links between high temperature and dryness with the relative abundance of C_4-species were found in North America (Teeri and Stowe, 1976; Stowe and Teeri, 1978), Europe (Pyankov et al., 2010), and Central Asia (Pyankov et al., 2000). High abundance of plants with C_4-type photosynthesis in arid climates is attributed to high water use efficiency, high photosynthetic temperature optimum, a stable quantum yield at high temperatures and other favorable physiological peculiarities (Black, 1971, 1973; Ehleringer, 1978; Ehleringer et al., 1997; Pyankov et al., 2000). Photosynthetic functioning of C_3-plants is tightly linked to spatial mesophyll structure in mature leaves (Laisk et al., 1970; Nobel and Walker, 1985; Terashima et al., 2001, 2011; Evans et al., 2009; Tosens et al., 2012; Flexas et al., 2013), therefore quantitative mesophyll parameters are considered to be good indicators of C_3-plants response to climate (Mokronosov, 1981; Pyankov and Mokronosov, 1993; Ivanova et al., 2018). Instead of individual parameters of cells or chloroplasts, the most promising traits are integral parameters of photosynthetic tissues as the total chloroplast number per leaf area (N_{chl}/A) and the total surface area of chloroplasts (A_{chl}/A) and cells (A_{mes}/A) per leaf area. These functional leaf traits were shown to predict the response of C_3-plants to climate in different climatic zones inside Russia-European steppes (Ivanova et al., 2018). The site-mean values of N_{chl}/A, A_{chl}/A and A_{mes}/A were higher in steppe than in boreal plants, and in C_3-plants growing in the desert steppe compared with those of the forest steppe. In contrast, C_4 plants in desert steppe showed low values of these mesophyll parameters. However, European steppe vegetation differs significantly from that of Asian semi-arid and arid regions in terms of species composition and vegetation characteristics (Lavrenko et al., 1991). Arid regions in the center of the Asian continent are characterized by a more continental climate with extreme cold and heat (Pyankov et al., 2000; Batima and Dagvadorj, 2000). The question as to whether leaf functional parameters respond similarly along aridity gradients in different parts of the continent is yet to be confirmed quantitatively.

Here we generalize analyses from our extensive data on leaf functional traits across a long and detailed latitudinal gradient – from forest steppe to hyper-arid desert – in Southern Siberia (Transbaikalia, Russia) and Mongolia. We hypothesized that mesophyll functional traits clearly indicate the response of plants with various leaf structure and photosynthetic performance along the aridity gradient in Central Asia. The aims of this study were 1) to find out which leaf traits among C_3- and C_4-plants, from whole-leaf to cell-level, respond to aridity, 2) to analyze the distribution of plant functional types (PFTs) along the aridity gradient and to define their influence on the site-mean values of leaf traits, 3) to reveal the role of mesophyll structure changes in photosynthetic performance of different PFTs along an aridity gradient.

2. Methods

2.1. Study sites

Studies were conducted in Russia and Mongolia along a 1600-km transect from Severobaikalsk (Transbaikalia in Southern Siberia, Russia) in the north to Ekhiin-Gol (Southern Mongolia) in the south (Fig. 1, Table 1). A transect was located in an area with predominantly sharply continental cryoarid climate. Transbaikalia and Mongolia are characterized by sharp contrasts in relief where latitudinal climate zones are mixed with elevation gradients. In our case, the elevation of studied regions varied from 400 to 2000 m however these elevations corresponded to the common elevation level of current landscape and represented plain sites which are flat areas with zonal soils and vegetation. We choose sites with characteristic zonal communities which would be appropriate for analysis along a latitudinal gradient. To allow the direct comparison of the studied sites we used universal and simple parameters for the assessment of climate. The climate of the study sites was characterized by the De Martonne aridity index (Ia) calculated using the equation: $Ia = MAP/(MAT + 10)$, where MAP is mean annual precipitation (mm) and MAT is mean annual temperature (°C). Temperature and precipitation data for the period 1980–2010 were taken from Climate Data Archives (Matsuura and Willmott, 2012a, b). Thirteen sites were selected for analysis along the transect. Overgrazed communities were avoided, however moderate grazing occurred in all sites. The transect passed through the different vegetation types – from steppe areas in the north to hyper-arid deserts in the south (Table 1). An increase in aridity, as the De Martonne aridity index decreased, was apparent along the studied transect.

2.2. Plant material

Vascular plant species which are quite common in the steppe zone of Southern Siberia and in the steppe and desert zones of Mongolia were used for this study. In total, 317 samples of 193 plant species were investigated in 13 sites (Table A.1 in Supplementary material). Plants

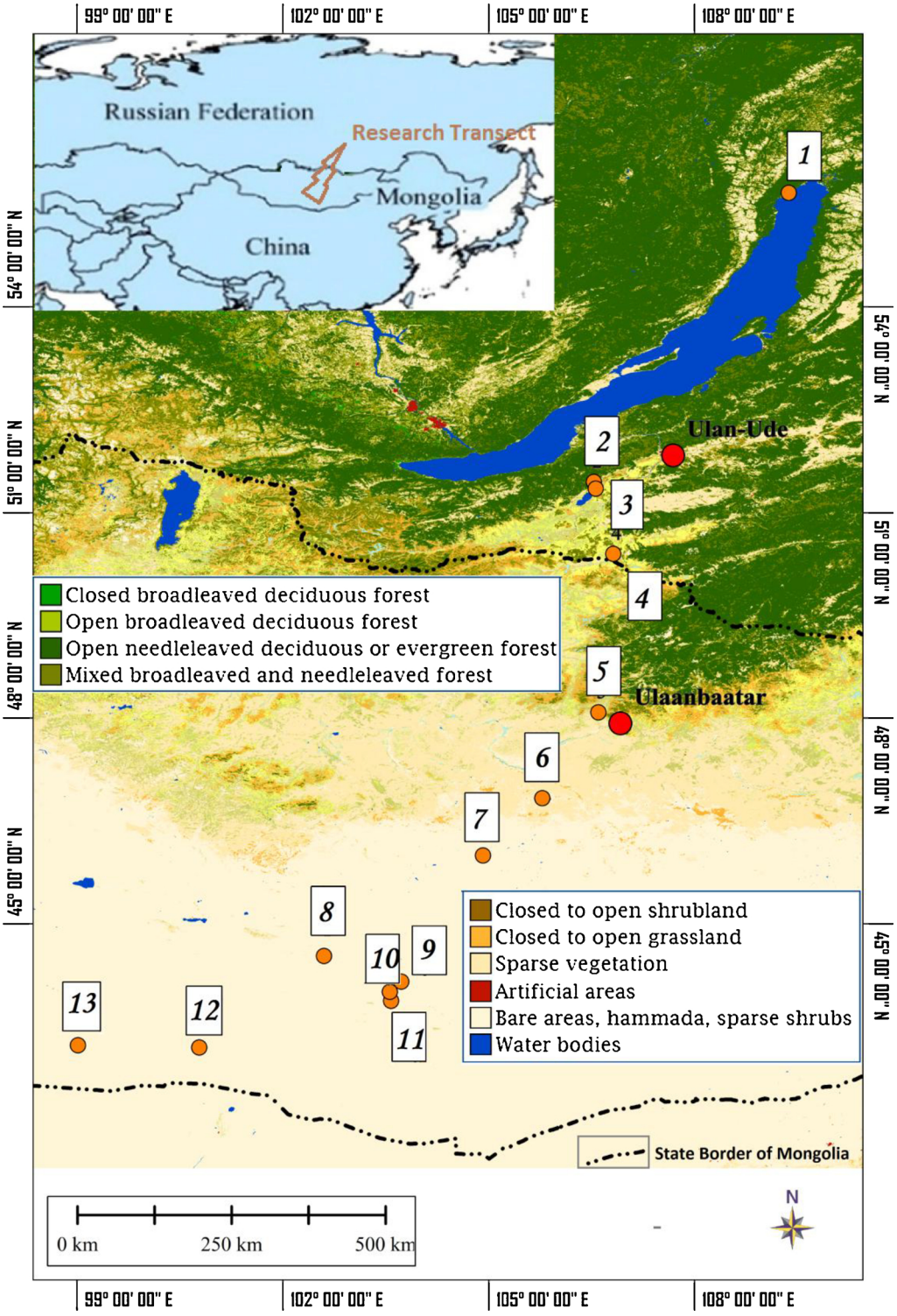

Fig. 1. Locations of the study sites on the map of GlobCover-2009 (according to Bontemps et al., 2010). Sites numbers are given according to Table 1.

selected were the dominant and most abundant species in each of the thirteen communities (Table 1), defined as comprising the main part of the projective foliage cover. Almost all species belonged to perennial plants – herbs, semishrubs, dwarf shrubs and shrubs. Species from 31 families were included in this study, the most dominant families being *Asteraceae* (28 species), *Fabaceae* (26 species), *Chenopodiaceae* (23 species) and *Poaceae* (19 species).

We classified the species under study according to the photosynthetic pathway, phylogeny, growth form and mesophyll anatomy into 9 plant functional types (PFTs): C_3-DH-DV – C_3-dicotyledonous herbs with dorsiventral mesophyll, C_3-DH-IP – C_3-dicotyledonous herbs with isopalisade mesophyll, C_3-DS-IP – C_3-dicotyledonous shrubs (semi-shrubs, dwarf-shrubs) with isopalisade mesophyll, C_3-M-Gr – C_3-monocotyledonous herbs with a graminoid leaf (*Poaceae, Cyperaceae*), Gymn – *Gymnosperms* (species of genus *Ephedra*), C_3-Succ – C_3-herbs and shrubs with succulent leaves (*Chenopodiaceae, Crassulaceae, Zygophyllaceae, Tamaricaceae, Alliaceae*), C_4-D-AK – C_4- dicotyledonous herbs and shrubs with atriplecoid and kochioid types of kranz-anatomy,

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Table 1
Geographical location, climatic and vegetation characteristics of the study sites. No. – site number, Elev, elevation (m); R, solar radiation incident on a horizontal surface (kWh m^{-2} d^{-1}); MAT, mean annual temperature (°C); MAP, mean annual precipitation (mm); Ia, De Martonne aridity index; n – number of studied species.

No.	Site (coordinates)	Type of community	Vegetation	Elev	R	MAT	MAP	Ia	n
1	Severobaikalsk (N 55°39′38″ E 109°22′22″)	Relict sites of meadow steppe in the forest zone	South taiga	506	3.04	−2.6	349	47.2	11
2	Jagodnoe (N 51°27′08 ″E 106°32′13″)	Grass-low herb petrophytic steppe	Mountain forest-steppe	669	3.42	−1.4	315	36.6	28
3	Tokhoi (N 51°21′07″ E 106°33′33″)	Grass-herb petrophytic steppe	Mountain forest-steppe	606	3.43	−1.1	249	28.0	38
4	Ust-Kiran (N 50°24′15″ E 106°49′31″)	Forb-sedge steppe	Meadow degraded steppe	590	3.55	−0.5	308	32.4	13
5	Partizan (N 48°04′43″ E 106°42′20″)	Shrub-forb-bunchgrass steppe	True steppe	1429	3.83	−0.2	244	24.9	22
6	Undzhul (N 46°49′55″ E 105°46′40″)	Shrub-bunchgrass steppe	Dry steppe	1373	4.13	1.5	199	17.3	13
7	Erdene-Dalai (N 45°59′36″ E 104°50′26″)	Shrub-onion-bunchgrass steppe	Dry steppe	1380	4.20	2.1	157	13.0	9
8	Arz Bogdo (N 44°32′ 19″ E 102°36′03″)	Shrub and semishrub-small bunchgrass petrophytic steppe	Mountain desert steppe	1764	4.36	3.5	160	11.9	14
9	Bulgan (N 43°52′54″ E 103°34′42″)	Shrub-forb- bunchgrass steppe	Mountain steppe	1720	4.43	3.2	118	8.9	11
10	Bulgan (N 44°00′45″ E 103°33′31″)	Semishrub-onion-small-bunchgrass steppe	Desert steppe	1442	4.41	4.2	117	8.2	24
11	Bayan Dzag (N 44°09′32″E 103°43′38″)	Desiccated coarse-shrub desert	True desert	1180	4.40	6.0	109	6.8	15
12	Gurvan-tes (N 43°12′20″ E 100°46′40″)	Semishrub-onion-small-bunchgrass steppe	Mountain desert steppe	2255	4.50	4.2	99	7.0	22
13	Ekhiin-Gol (N 43°14′29″ E 99°0′51″)	Sparse shrub desert	Hyper-arid desert	985	4.69	8.5	58	3.1	9

C_4-M-Pan – C_4-monocotyledonous herbs with panicoid type of kranz-anatomy (*Poaceae*), C_4-D-Sals – C_4-dicotyledonous herbs and shrubs with salsoloid type of kranz-anatomy. Only two species among studied plants exhibit Crassulacean Acid Metabolism (CAM) – *Orostachys spinosa* and *Orostachys malacophylla* (Tsendeekhuu and Black, 2005). We classified different species of *Alliaceae* to C_3-Succulents as suggested in Gamalei (1988) because of the similarity of their leaf and mesophyll traits with dicotyledonous succulent species. The succulence was determined as leaves or green aphyllous stems with a high leaf water content (> 80%) and a large leaf / stem thickness (700–1000 μm and more). The types of leaf anatomy in C_4-plants were determined according to previous studies (Carolin et al., 1975; Voznesenskaja and Gamalei, 1986).

According to classification defined vegetation zone / type, plant species were classified into groups, named as zonal vegetation types: forest-steppe, steppe, desert-steppe and desert species. Steppe and desert plants were classified into subgroups: steppe and montane-steppe, desert and montane-desert. The species studied have been included in zonal vegetation types mostly according to Peshkova (2001). In order to classify some species not recorded by Peshkova (2001) other sources were involved (Grubov, 1982; Malyshev and Peshkova, 1984; Ulziykhutag, 2003; Urgamal et al., 2014). In a few cases the authors mentioned above interpreted species groupings differently. We adopted those species that were consistent with our own knowledge and field experience. Those species are indicated by double-asterisks at the column with “Classification to zonal vegetation type” in Table A.1 (Supplementary material).

Plant material was sampled in Southern Siberia in July and in Mongolia from the second half of July to the middle of August that coincided with the peak of zonal vegetation development after the period of maximal precipitation. Leaves were harvested from 10 to 20 fully developed individuals of each species (3–5 leaves from the middle leaf tier per individual). Fresh leaf material was collected and stored in a refrigerator in wet filter paper for 2–3 h. From ten to twelve leaves per species (one leaf per individual) were used to determine leaf area and thickness, leaf mass per area and leaf dry matter content, 9–12 leaves were taken for measuring chlorophyll and carotenoid content, and 20–30 leaves were fixed in tubes in a solution of glutaraldehyde (3.5% glutaraldehyde in 0.15 M phosphate buffer, pH 7.4) for anatomical measurements. Physiological and biochemical parameters were measured in a lesser number of samples with n = 110 than the parameters of leaf structure with n = 315.

2.3. Leaf traits

We investigated functional traits from whole leaf to mesophyll, cell and chloroplast (see list of leaf traits and abbreviations in Table A.2 (Supplementary material). Leaf area (LA, cm^2) was measured using a digital camera and the Simagis Mesoplant™ analyzer (SIAMS, Russia, Ekaterinburg) for 10–12 leaves per species. To determine the leaf mass per area (LMA, g m^{-2}) and the leaf dry matter content (LDMC, %) we used leaf discs of known diameter from the central part of the leaf or whole leaf blades in the case of small or narrow leaves. These were photographed, weighed wet, dried at 75 °C for 48 h and weighed again after drying. In the case of plants with small cylinder-like leaves or aphyllous plants (species of *Ephedra*, *Haloxylon ammodendron, Calligonum mongolicum*) we calculated leaf parameters per area unit of photosynthetic organ projection.

Quantitative parameters of photosynthetic tissues were determined according to the method of Mokronosov (1981) described in detail in Pyankov et al. (1998) and Ivanova and Pyankov (2002) using analyses of leaf cross-sections and leaf tissue maceration. Then 20–30 μm thick leaf cross-sections from 10 leaves per species (one leaf per individual) were produced with a freezing microtome (Microm, Carl Zeiss, Germany), visualized with a light microscope (Axiostar plus, Zeiss, Germany) on a computer display and analyzed by the image analysis system SIAMS Mesoplant (SIAMS, Yekaterinburg, Russia). An analysis of leaf cross-sections was performed for the measuring of leaf thickness (LT), relative volume of mesophyll tissue (RV_{mes}, %) in the leaf (with intercellular airspaces) and chloroplast sizes. RV_{mes} was evaluated in 10 replicates per sample (two cross-sections per leaf, 5 leaves per species, one leaf per individual). Chloroplast projection area and perimeter were measured on leaf cross-sections in 30 replicates per sample (3 chloroplasts per one cross-section, two cross-sections per leaf, 5 leaves

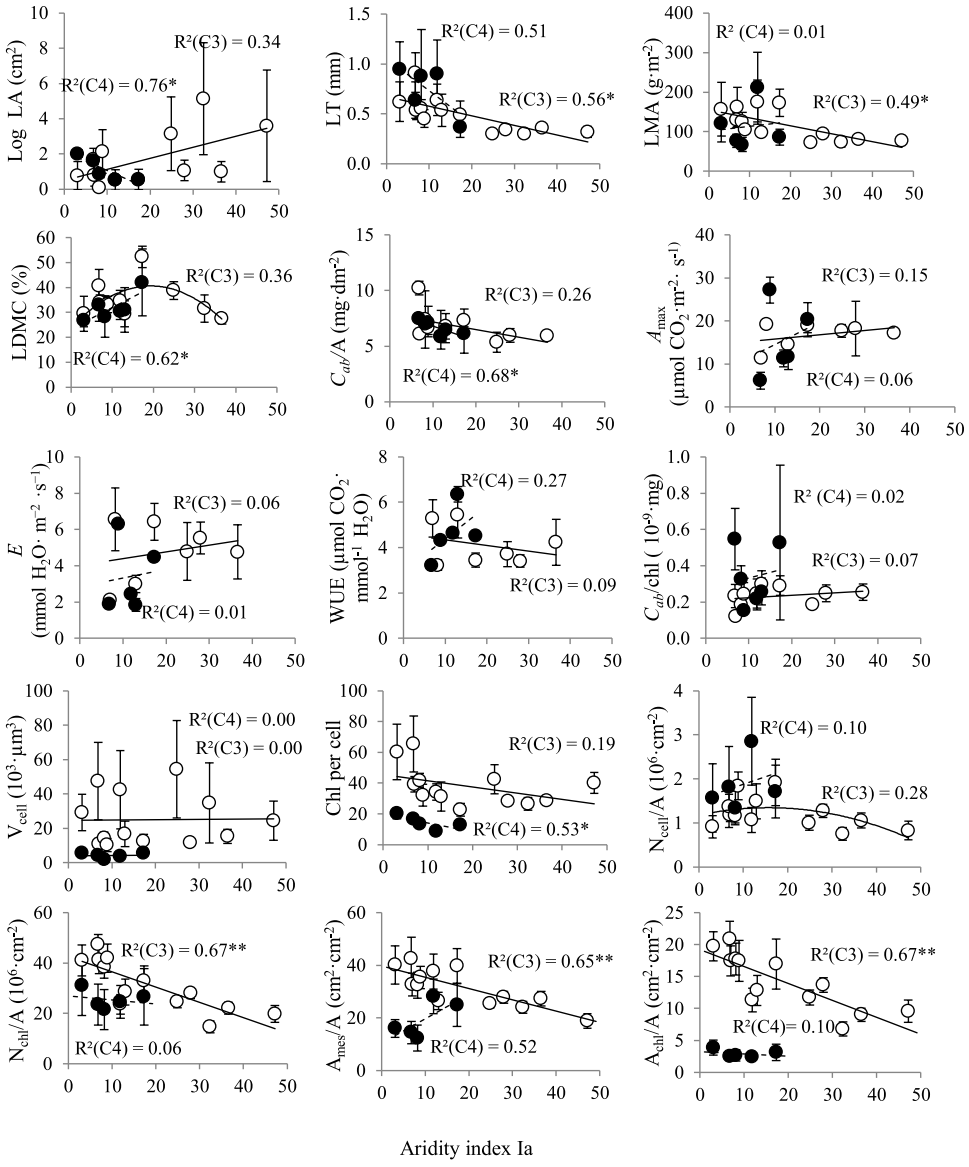

Fig. 2. Changes in site-mean leaf traits of C_3- (open dots) and C_4-plants (full dots) along an aridity gradient in Southern Siberia and Mongolia. A regression line between site-means and aridity index is shown, full line: for C_3-plants, dashed line: C_4-plants. R^2 – coefficient of determination for C_3- (C3) and C_4-plants (C4), significance levels of correlation coefficient *, $p \leq 0.05$; **, $p \leq 0.01$.

per species, one leaf per individual). Cell sizes were determined in cell suspension after maceration of leaf pieces in 1 N HCl under heating up to 40–50 °C in 30 replicates per sample (5 leaf pieces from the middle parts of leaf blades, one piece per leaf, and one leaf per individual). Cell projection area and perimeter were measured separately for each type of photosynthetic tissue (palisade and spongy mesophyll for C_3-plants, mesophyll and bundle sheath cells for C_4-plants, simple and segmented mesophyll cells for grasses) with a light microscope (Axiostar plus, Zeiss, Germany) and the image analysis system SIAMS Mesoplant (SIAMS, Yekaterinburg, Russia). The mesophyll cell volume (V_{cel}, 10^3 μm^3) and the chloroplast volume (V_{chl}, μm^3) as well as their surface areas (S_{cel}, 10^3 μm^2, and S_{chl}, μm^2) were calculated by the projection method described in detail by Ivanova and Pyankov (2002) and Ivanova et al. (2018). Chloroplast number per cell (Chl per cell) was determined in 30 replicates per sample in the same cells that were measured for cell sizes.

The number of cells per leaf area unit (N_{cel}/A, 10^6 cm^{-2}) was evaluated in 20 replicates in cell suspension using a hemocytometer ("Goryaev chamber", Minimed, Bryansk, Russia) after maceration of leaf pieces of known area in 2–3 ml of 20% KOH after heating them to 90 °C (5 leaf pieces from the middle parts of leaf blades, one piece per leaf, and one leaf per individual). The chloroplast number per leaf area (N_{chl}/A, 10^6 cm^{-2}) was calculated by multiplying the chloroplast number per cell and the cell number per leaf area for palisade and spongy mesophyll, or mesophyll and bundle sheath cells, or for the whole mesophyll in the case of uniform or irregular cells.

The total cell / chloroplast surface area per leaf area unit (A_{mes}/A and A_{chl}/A) was determined by multiplying the average cell / chloroplast surface area and the cell / chloroplast number per leaf area. We determined also the area of cell surface covered by chloroplasts (k) using the equation: $k = (A_{chl}/A)/(2A_{mes}/A)$. A_{mes}/A and A_{chl}/A describe the surface areas through which CO_2 penetrates from the intercellular air space to the sites of Rubisco carboxylation in the chloroplast. In C_3-plants this process occurs similarly in cells of both palisade and spongy mesophyll. Therefore, in the case of C_3-species A_{mes}/A and A_{chl}/A were separately calculated for palisade and spongy cells and then summarized. In the case of C_4-plants, mesophyll and bundle sheath cells and chloroplasts in them are functionally different, because diffusion from the intercellular spaces in the cell cytosol and the initial carboxylation by phosphoenolpyruvate carboxylase (PEPC) occur only

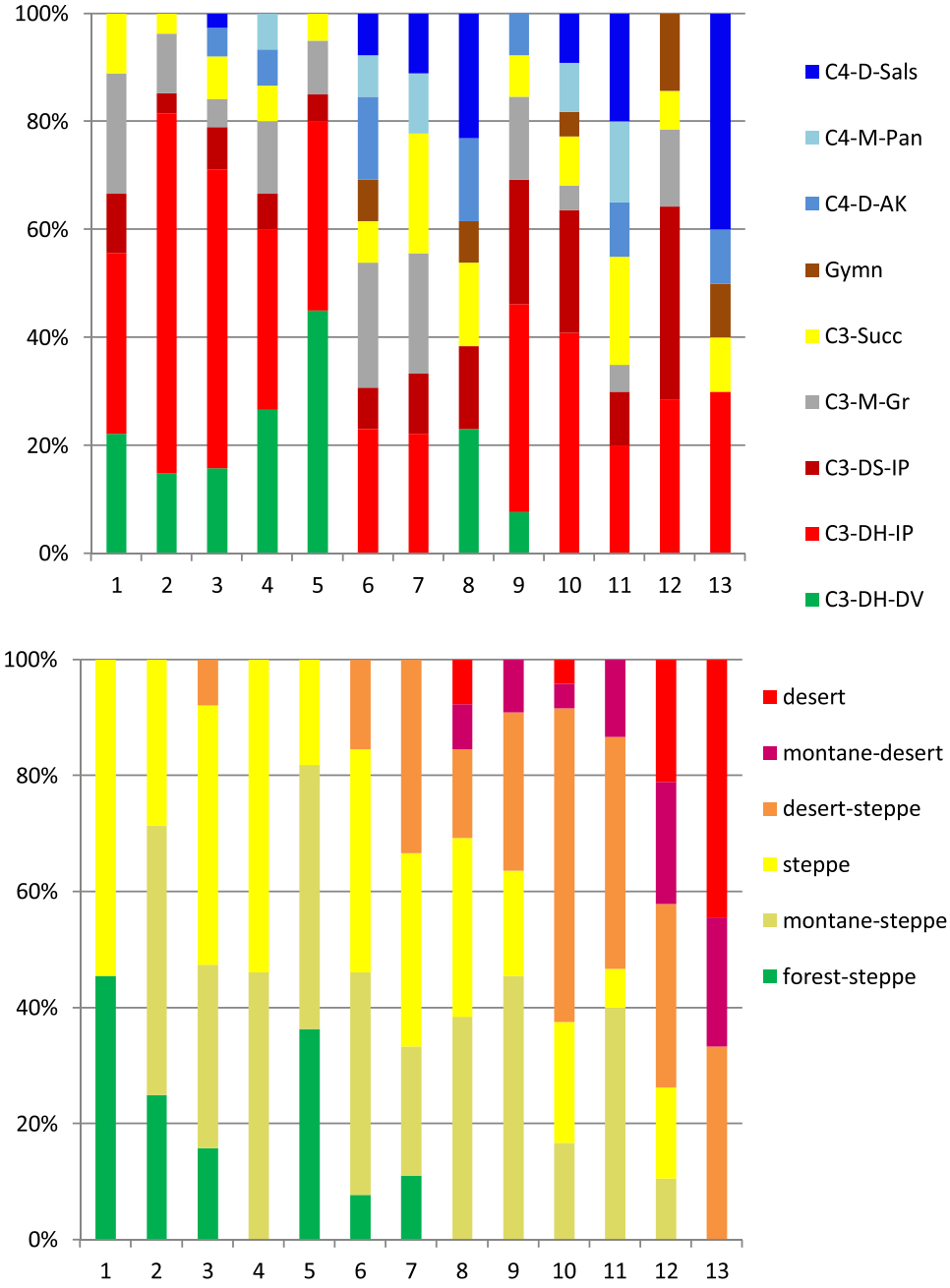

Fig. 3. Distribution of PFTs (A) and zonal vegetation types (B) among studied species in study sites. Y-axis presents the percentage of species in the total number of plant species studied within a site. X-axis presents site numbers from 1 to 13 according to Table 1. C_3-DH-DV – C_3-dicotyledonous herbs with dorsiventral mesophyll, C_3-DH-IP – C_3-dicotyledonous herbs with isopalisade mesophyll, C_3-DS-IP – C_3-dicotyledonous shrubs with isopalisade mesophyll, C_3-M-Gr – C_3-monocotyledonous herbs with graminoid type of mesophyll, C_3-Succ – C_3-herbs and shrubs with succulent leaves, Gymn – Gymnosperms (*Ephedra*), C_4-D-AK – C_4-dicotyledonous herbs and shrubs with atriplecoid and kochioid types of kranz-anatomy, C_4-M-Pan – C_4-monocotyledonous herbs with panicoid type of kranz-anatomy, C_4-D-Sals – C_4-dicotyledonous herbs and shrubs with salsoloid type of kranz-anatomy.

in the mesophyll cells, while carbon dioxide fixation is performed only in the chloroplasts of the bundle sheath cells. For this reason, we calculated A_{mes}/A for C_4-plants as the surface area of mesophyll cells only and A_{chl}/A as the surface area of the chloroplasts of bundle sheath cells only.

To determine the content of chlorophyll $a + b$ per leaf area (C_{ab}/A) pigments were immediately extracted with 80% acetone from fresh leaf pieces and measured using the Odyssey DR/2500 portable spectrophotometer ("HACH", USA). C_{ab}/A was calculated according to Lichtenthaler and Wellburn (1983). The chlorophyll $a + b$ content per chloroplast (C_{ab}/chl, 10^{-9} mg) was derived by dividing the pigment content per unit leaf area (C_{ab}/A) by the chloroplasts number per unit area (N_{chl}/A).

Light-saturated photosynthetic rate per leaf area unit (A_{max}) and transpiration rate (E) were determined in the first half of the day – from 9:00 till 14:00 – depending on the daily photosynthetic activity peak of the species. The measurements were carried out on intact mature leaves of three to five individuals per species using an infrared gas analyzer Li-6400xt (Li-COR, USA) under ambient CO_2 concentration, controlled leaf temperature (24 °C) and photosynthetic photon flux density (PPFD) of 1500 µmol photons m^{-2} s^{-1} for C_3-species and 2000 µmol photons m^{-2} s^{-1} for C_4-species. The photosynthetic activity of the chloroplast (A_{max}/chl, 10^{-10} µmol CO_2 s^{-1}) was calculated by dividing the photosynthetic rate per unit leaf area (A_{max}) by the number of chloroplasts per leaf area (N_{chl}/A). The rate of CO_2-transfer through mesophyll surface area (TR_{mes}) and chloroplast surface area (TR_{chl}) were calculated by dividing the maximum rate of CO_2-uptake per leaf area (A_{max}) by A_{mes}/A or A_{chl}/A, respectively.

2.4. Statistical analysis

For some traits, such as leaf area or cell volume, the data were log transformed to meet the assumption of normality. The coefficient of determination was used to reveal the relationship between aridity index

and leaf parameters. To test for differences between categories (C_3- versus C_4-plants, PFTs and vegetation types) following one-way ANOVAs we used Tukey-post hoc tests, and in appropriate cases a *t*-test was used. Differences were considered to be significant at $p \leq 0.05$. All statistical analyses were carried out in Statistica 6.0 (StatSoft Inc.).

3. Results

Initial analysis of site-mean values across all species in the study failed to reveal changes in leaf traits except an increase in leaf thickness (LT) and leaf mass per area (LMA) with a decrease in aridity index (Ia). Based on the separate analysis of C_3- and C_4-plants, we found significant trends in five out of more than 20 leaf parameters for C_3 plants along the aridity gradient (Fig. 2). All parameters that showed strong relationships between site-means and aridity belonged to the characteristics of leaf structure. The strongest correlations with climate were found for integrated mesophyll parameters per leaf area – chloroplast number (N_{chl}/A), the total surface area of mesophyll (A_{mes}/A) and chloroplasts (A_{chl}/A). These mesophyll traits substantially increased with a decrease in Ia, indicating the increasing aridity along the latitudinal gradient. Among whole-leaf traits, LT and LMA also increased with aridity in C_3-species. Variation in the leaf area (LA) and leaf dry matter content (LDMC) could not be reliably explained by climate. Within a site, different C_3-species showed widely ranging values of cell volume and chloroplast number per cell (Fig. 2), that showed no patterning along the gradient. Within C_3-plants, the cell number per leaf area varied to a lesser degree than cell size, while in C_4-plants it was reversed. Physiological and biochemical parameters such as photosynthesis and transpiration rates, water use efficiency and pigment content did not show tendencies to change with aridity. Contrary to C_3-plants, C_4-species were abundant only at the southern end of the gradient, where there was an increase in LA, chlorophyll $a + b$ content per leaf area and chloroplast number per cell and a decrease in LDMC and A_{mes}/A in C_4-plants.

We found common features as well as changes in the distribution of PFTs among all species in the study along the aridity gradient (Fig. 3A). C_3-dicot herbs and shrubs with isopalisade mesophyll dominated across the entire gradient. The proportion of C_3-dicots with dorsiventral mesophyll was higher at the northern part of the gradient and this PFT disappeared in southern sites. The proportion of C_4-plants, especially C_4-salsoloid type, increased with aridity. A clear tendency was also found in the distribution of zonal vegetation types of plants (Fig. 3B). Forest-steppe species were presented in the northern half of the gradient whereas desert-steppe plants appeared at the values of Ia less than 13. Desert species dominated at the southern end of the gradient with values of Ia from 3 to 7.

The main differences between PFTs consisted of LT, LMA and mesophyll proportion in the leaf (Fig. 4). C_3-succulents, C_4-salsoloids and gymnosperms (species of genus *Ephedra*) had two- to three-fold higher LT than all other PFTs. C_3-dicots are characterized by the largest values of the relative volume of mesophyll (RV_{mes}) which reached 70–80% of the leaf. In the case of C_3-grasses, *Ephedra* type and C_4-dicots, RV_{mes} was lesser – 50–60%. Minimal values of RV_{mes} were found for C_4-Salsoloid type – 35–40%. Very high values of LMA were intrinsic for *Ephedra* and C_4-Salsoloid type. We found significant differences in cell size or chloroplast number per cell for C_3-succulents which had 4–5-fold larger cells with a huge amount of chloroplasts in them than other PFTs. Leaf traits of two CAM-species *Orostachys spinosa* and *O. malacophylla* were similar to C_3-succulents with high water content – 95% in both species, thick leaves – 1930 μm in *O. spinosa* and 960 μm in *O. malacophylla* but had much larger mesophyll cells – $3.8 \cdot 10^6$ μm^3 and $1.1 \cdot 10^6$ μm^3 accordingly, and lower A_{chl}/A – 6.7 and 7.5. Integrated mesophyll parameters mostly differed between C_3 and C_4-plants with lower values in the latter. Among C_3-plants, N_{chl}/A and A_{mes}/A were higher in isopalisade species, graminoids, succulents and gymnosperms than those in plants with dorsiventral mesophyll.

The differences in leaf structure between zonal vegetation types can be seen in Fig. 5. Plants with C_4-photosynthesis did not show any clear difference in leaf parameters between steppe, desert-steppe and desert species. In the case of C_3-plants, desert and desert-steppe species differed from steppe and forest-steppe plants by higher N_{chl}/A and A_{chl}/A. In addition, montane-desert plants possessed the thickest leaves with the largest values of cell volume and chloroplast number per cell.

Physiological and biochemical data show comparable rates of photosynthesis and transpiration per leaf area unit between different PFTs as well as between different zonal vegetation types (Table 2). However, PFTs were distinguished by maximum photosynthetic activity per chloroplast and CO_2-transfer rate per mesophyll (TR_{mes}) and chloroplast (TR_{chl}) surface with maximal values in C_4-plants and minimal values in gymnosperms. Zonal vegetation types were characterized by different water use efficiency (WUE) and pigment forms ratio. Compared to forest-steppe and steppe species, desert-steppe C_3-plants had a 1.5-fold increase in WUE. At the same time, desert-steppe C_3-plants did not differ from steppe either in LT or LMA, but exhibited higher N_{chl}/A and A_{chl}/A as well as a larger share of cell surface occupied by chloroplasts (k) (Fig. 5). Desert plants possessed lower values of chlorophyll *a/b* and a higher chlorophyll/carotenoid ratio than other zonal vegetation types (Table 2).

The analysis of mesophyll structure for PFTs and zonal vegetation types allowed us to generalize the structural and functional diversity of studied plants in a common scheme (Fig. 6). A combination of PFTs and vegetation types is described by points on a plane with two main traits as axes. Since cell volume and A_{chl}/A belong to the key parameters of mesophyll structure, contributing to the main differences in photosynthetic tissues anatomy between PFTs and vegetation types, one axis on a plane is represented by mesophyll cell volume, another axis is expressed by A_{chl}/A. When plant groups were more finely separated by PFT and zonal vegetation type, we found local differences in cell volume and integrated parameters of mesophyll. For example, the cells of steppe plants in C_3-dicot shrubs with IP type were twice as small as those of desert-steppe (*t*-test, $p < 0.001$). Among steppe plants the cell volume of C_3-dicot herbs was on average three-times larger than that in C_3-shrubs (*t*-test, $p < 0.05$).

4. Discussion

4.1. The changes of leaf functional traits along an aridity gradient

We investigated a big data set on leaf functional traits along an extensive latitudinal gradient that allowed us to test our hypothesis about the indicative significance of leaf functional traits for plant response to climate in semi-arid and arid regions of Central Asia. Our results demonstrated the differing relevance of leaf traits as response indicators among PFTs. Some leaf traits known to be sensitive to climate, as in our case, were not related to aridity. Globally, site-mean leaf size typically scales with water availability and temperature (Givnish, 1984; Royer et al., 2005; Peppe et al., 2011). Leaf size, shape, and teeth are widely claimed in paleoclimate reconstructions as good predictors of environmental variables (Royer et al., 2005; Peppe et al., 2011). However, such an approach mostly concerns biomes dominated by woody dicots. The percentage of herbs and monocots (especially grasses, sedges, and onions among them) is very high in semi-arid and arid areas of Central Asia, where strong aridity stress leads to convergent leaf traits in particular simple shape and small size with a reduction of the leaf lamina in some species. Therefore trait–environment studies require other traits instead of leaf size and shape.

An increase in LMA and LT among both C_3- and C_4-plants with dryness as well as a weak tendency for LDMC of C_3-plants was found in many cases (Fonseca et al., 2000; Niinemets, 2001; Lavorel et al., 2008; Prentice et al., 2011). In our previous study in European Russian steppes, no differences in LMA and LT were found in C_3-plants between forest steppe and desert steppe, which were twice as diverse in the

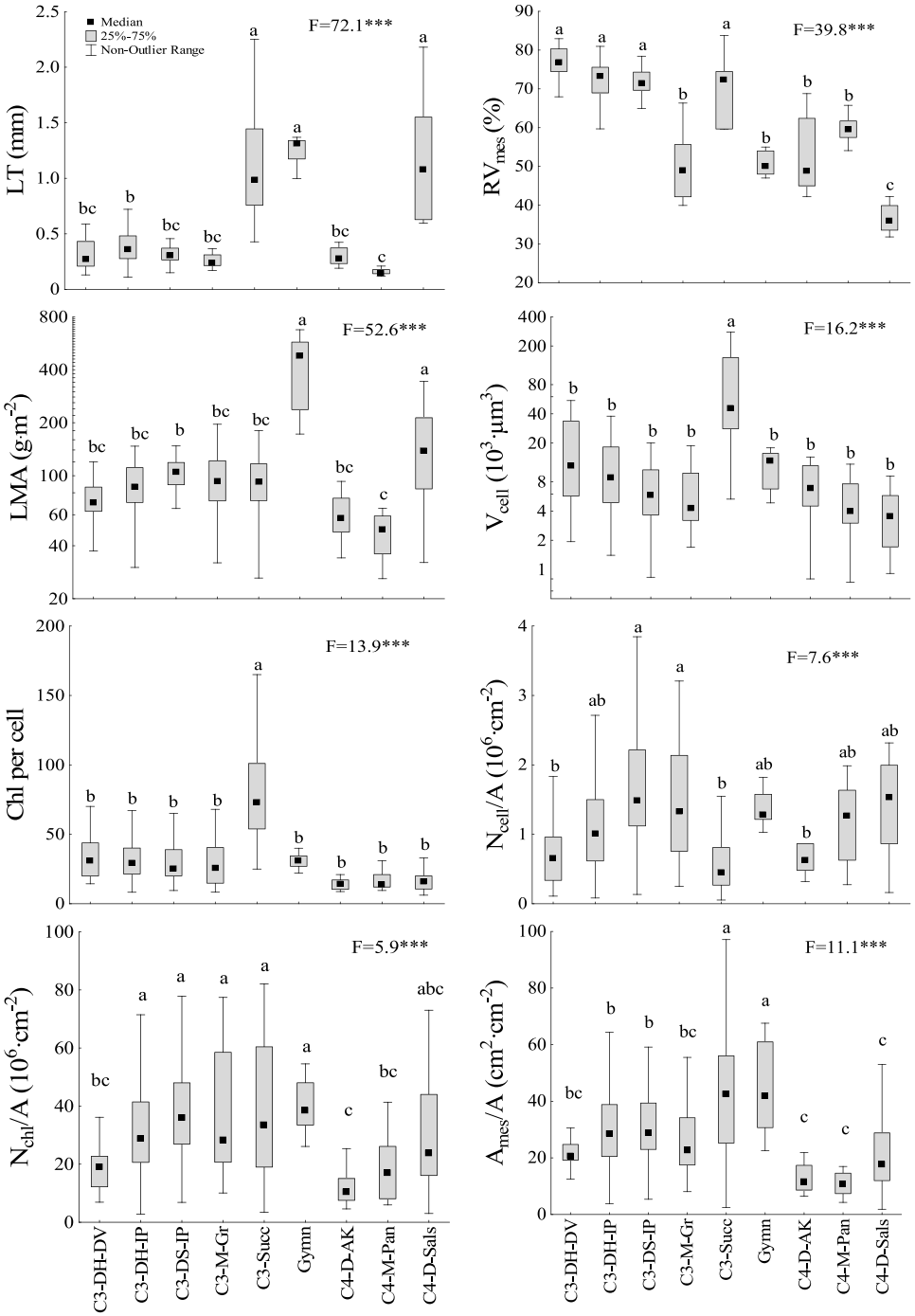

Fig. 4. Distribution of leaf traits for plants of different plant functional types (PFTs). C_3-DH-DV – C_3-dicotyledonous herbs with dorsiventral mesophyll, C_3-DH-IP – C_3-dicotyledonous herbs with isopalisade mesophyll, C_3-DS-IP – C_3-dicotyledonous shrubs with isopalisade mesophyll, C_3-M-Gr – C_3-monocotyledonous herbs with graminoid type of mesophyll, C_3-Succ – C_3-herbs and shrubs with succulent leaves, Gymn – Gymnosperms *(Ephedra)*, C_4-D-AK – C_4-dicotyledonous herbs and shrubs with atriplecoid and kochioid types of kranz-anatomy, C_4-M-Pan – C_4-monocotyledonous herbs with panicoid type of kranz-anatomy, C_4-D-Sals – C_4-dicotyledonous herbs and shrubs with salsoloid type of kranz-anatomy. The results of one-way ANOVA are presented: F-criteria and *p*-level. *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$, ns – not significant.

aridity index (Ivanova et al., 2018). We found a general increase in LT and LMA among C_3-plants along the extensive aridity gradient investigated in this study (Fig. 2); however, these trends reflected the general differences between vegetation zones. Two groups of the sites studied within the gradient could be distinguished. The first group unites site-mean values from forest steppe to true steppe with LT 300–350 µm and LMA 70–90 g m^{-2}, and the second group at the other part of the gradient brings together sites from dry steppe to desert where LT reaches more than 500 µm and LMA is more than 100 g m^{-2}. Notably, most C_4-species manifested the highest values of these parameters in sites at the southern end of the transect. Differences occurring in LT and LMA between PFTs were higher than those between sites along the aridity gradient (Fig. 4). This finding confirms the predominant effect of PFT on LMA and LT variation (see also Castro-Diez et al., 2000; Poorter et al., 2009; Ivanova et al., 2018).

The strongest relationship with the aridity index across the entire gradient was found for mesophyll traits (Fig. 2). The fact that site-mean values of N_{chl}/A, A_{mes}/A and A_{chl}/A among C_3-plants vary in response to aridity indicates that species having low values of integrated mesophyll traits are replaced by species with high values in a more arid climate. At the same time the cell-level mechanism of these traits regulation represents a trade-off between cell size and cell number depending on functional peculiarities and evolutionary history of the species. Remarkably, the maximal rate of photosynthesis has comparable site-mean values in all sites of the gradient. The occurence of C_4-plants being restricted to a much shorter latitudinal gradient could influence the character of relationships between leaf traits and aridity. However we suppose that biochemical peculiarities which contribute to mesophyll conductance

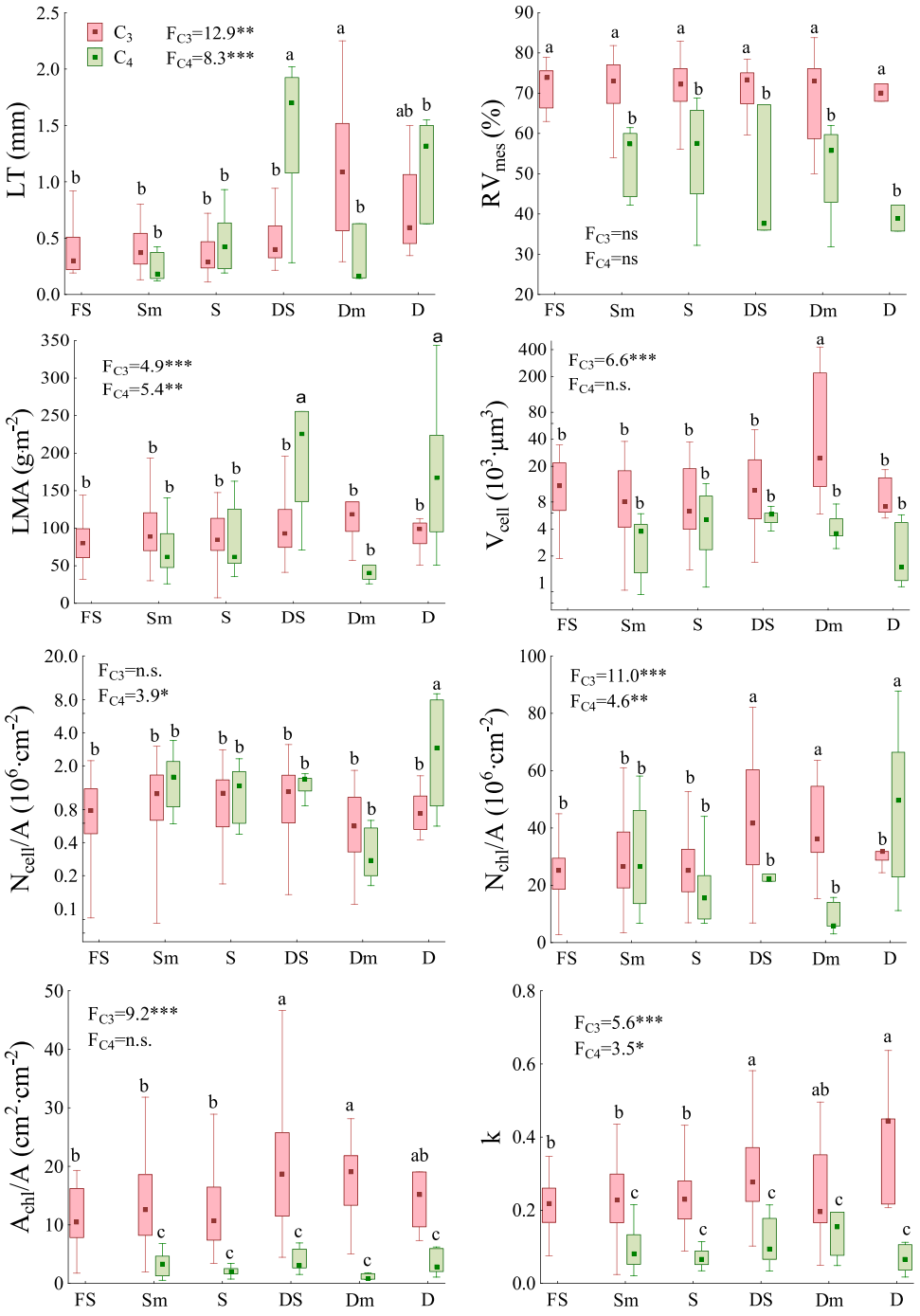

Fig. 5. Differences in leaf traits between plant species belonging to different zonal vegetation types inside C_3-plants (red box plots) and C_4-plants (green box plots). X-axis represents zonal vegetation types: FS – forest-steppe species, Sm – montane-steppe, S – steppe, DS – desert-steppe, Dm – montane-desert, D – desert species. The results of one-way ANOVA separately for C_3 and C_4-plants are presented: F- criteria and *p*-level. *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$, ns – not significant (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

(g_m) in C_4-plants may also lead to changing the trait–climate relationships for these plants in comparison to C_3-species (see below).

It is well known that dryness adversely affects the photosynthetic process in plants (Flexas et al., 2004; Chaves et al., 2009). However, A_{max} has even been shown to increase with site aridity in a global multi-species analysis (Maire et al., 2015). Photosynthetic traits can be influenced not only by climate but also by soil properties such as soil pH, available phosphorus, nitrogen and others. Considering this circumstance we excluded all saline as well as disturbed sites from our analysis since the leaf traits of halophyte or ruderal species as well their response to climate should be subjects of special studies. In our case, we found the constancy of site-mean A_{max} along the aridity gradient that can be achieved by replacement of PFTs related to leaf structure and functioning. A large within-site variation in most physiological parameters was observed in all parts of the gradient. This variation increased to the most arid end of the gradient (Fig. 2) and can be explained by diversity of PFTs in every site (see below).

4.2. Functional diversity of plants and their change along an aridity gradient

Patterns of C_3- and C_4-plant leaf types across semi-arid and arid

Table 2
Leaf functional traits of plant species belonging to different plant functions types (PFTs) and zonal vegetation types. C_3-DH-DV – C_3-dicotyledonous herbs with dorsiventral mesophyll, C_3-DH-IP – C_3-dicotyledonous herbs with isopalisade mesophyll, C_3-DS-IP – C_3-dicotyledonous shrubs with isopalisade mesophyll, C_3-M-Gr – C_3-monocotyledonous herbs with graminoid type, Gymn – Gymnosperms, C_3-Succ – C_3-herbs and shrubs with succulent leaves, C_4-D-AK – C_4-dicotyledonous herbs and shrubs with atriplecoid and kochioid types of kranz-anatomy, C_4-M-Pan – C_4-monocotyledonous herbs with panicoid type of kranz-anatomy, C_4-D-Sals – C_4-dicotyledonous herbs and shrubs with salsoloid type of kranz-anatomy. N1 – samples number for A_{chl}/A and other mesophyll parameters, N2 – samples number for physiological traits. Samples averaged means and standard errors are given (data for Gymn-type is averaged only for two samples), nd – no data. F – F- criteria of one-way ANOVA and p-level. *, $p \le 0.05$; ***, $p \le 0.001$, ns – not significant. A_{chl}/A – the total chloroplast surface area per leaf area unit, $cm^2\ cm^{-2}$; A_{max} – maximal rate of photosynthesis per leaf area, $\mu mol\ m^{-2}\ s^{-1}$; E – transpiration rate per leaf area, $mmol\ m^{-2}\ s^{-1}$; WUE – water use efficiency, $\mu mol\ mmol^{-1}$; TR_{mes} – rate of CO_2-transfer through the mesophyll surface, $\mu mol\ m^{-2}\ s^{-1}$; TR_{chl} – rate of CO_2-transfer through the chloroplast surface, $\mu mol\ m^{-2}\ s^{-1}$; Cab/chl – chlorophyll $a + b$ content per chloroplast, 10^{-9} mg; A_{max}/chl – maximal rate of photosynthesis per chloroplast, $10^{-10}\ \mu mol\ CO_2\ s^{-1}$; a/b – chlorophyll a/b ratio, $g\ g^{-1}$; chl/car – chlorophylls/carotenoids ratio, $g\ g^{-1}$.

Group	N1	N2	A_{chl}/A	A_{max}	E	WUE	TR_{mes}	TR_{chl}	Cab/chl	A_{max}/chl	a/b	chl/car
Plant functional types (PFTs)												
C_3-DH-DV	34	6	9 ± 1b	12 ± 2b	3.9 ± 0.3a	3.1 ± 0.3b	0.6 ± 0.1b	1.8 ± 1.0b	0.26 ± 0.05ab	0.9 ± 0.4b	1.8 ± 0.2a	5.0 ± 0.6a
C_3-DH-IP	116	38	15 ± 1a	15 ± 1b	5.3 ± 0.7a	3.3 ± 0.3b	0.6 ± 0.1b	1.2 ± 0.2b	0.22 ± 0.02b	0.6 ± 0.1b	1.8 ± 0.1a	5.0 ± 0.4a
C_3-DS-IP	36	14	17 ± 1a	22 ± 4a	6.7 ± 3.6a	3.6 ± 0.3ab	0.8 ± 0.1b	1.6 ± 0.3b	0.21 ± 0.03b	0.7 ± 0.1b	1.9 ± 0.1a	5.5 ± 0.4a
C_3-M-Gr	29	18	15 ± 2ab	15 ± 2b	4.3 ± 0.6a	4.0 ± 0.3ab	0.6 ± 0.1b	1.5 ± 0.3b	0.21 ± 0.03b	0.6 ± 0.1b	1.8 ± 0.1a	5.8 ± 0.3a
C_3-Succ	37	14	20 ± 2a	24 ± 4a	6.0 ± 2.1a	5.4 ± 1.2a	0.5 ± 0.1b	1.1 ± 0.2b	0.28 ± 0.04ab	0.6 ± 0.1b	1.6 ± 0.1a	6.0 ± 0.8a
Gymn	12	2	16 ± 1ab	12 ± 2b	3.4 ± 1.0a	3.5	0.2 ± 0.0c	0.6 ± 0.0c	0.26 ± 0.00ab	0.2 ± 0.04c	2.2	4.0
C_4-D-AK	15	6	2 ± 0.5c	18 ± 4ab	3.8 ± 1.0a	4.7 ± 0.2a	0.7 ± 0.1ab	5.2 ± 1.4a	0.48 ± 0.16a	3.1 ± 0.8a	1.7 ± 0.1a	5.6 ± 0.4a
C_4-M-Pan	14	4	2 ± 0.4c	10 ± 2b	1.5 ± 0.3a	6.4 ± 0.6a	1.3 ± 0.3a	3.0 ± 0.2a	0.22 ± 0.04ab	0.8 ± 0.2b	2.3 ± 0.1a	4.4 ± 0.3a
C_4-D-Sals	22	8	3 ± 0.4c	10 ± 3b	2.1 ± 0.5a	4.8 ± 1.5a	1.1 ± 0.2a	2.1 ± 0.1ab	0.31 ± 0.06ab	0.9 ± 0.2b	1.7 ± 0.2a	5.1 ± 1.4a
F (PFT)	315	110	15.3***	ns	ns	ns	2.0*	6.0***	2.4*	9.4***	ns	ns
Zonal vegetation types inside C_3-plants												
Forest-steppe	34	14	12 ± 1b	16 ± 2a	4.9 ± 0.8a	3.5 ± 0.3b	0.5 ± 0.06	1.3 ± 0.3	0.24 ± 0.04	0.6 ± 0.1	1.7 ± 0.1a	5.5 ± 0.7b
Steppe	147	51	13 ± 1ab	17 ± 1a	5.5 ± 0.6a	3.4 ± 0.2b	0.7 ± 0.07	1.5 ± 0.2	0.22 ± 0.02	0.6 ± 0.1	1.7 ± 0.1a	5.3 ± 0.3b
Desert-steppe	62	21	20 ± 1a	20 ± 3a	4.9 ± 1.3a	5.0 ± 0.7a	0.5 ± 0.1	1.0 ± 0.2	0.21 ± 0.03	0.5 ± 0.1	1.9 ± 0.1a	5.1 ± 0.2b
Desert	20	5	18 ± 2a	nd	nd	nd	nd	nd	0.35 ± 0.07	nd	1.3 ± 0.1b	8.4 ± 1.5a
F (Zonal veg.types)	263	91	14.3***	ns	ns	5.7***	ns	ns	ns	ns	2.4*	2.0*

regions in Central Asia have been generalized on a plane of mesophyll parameters as axes (Fig. 6). One axis represented by mesophyll cell volume was in our study in higher degree linked to PFTs, another axis expressed by A_{chl}/A was found to be correlated with climate aridity. A similar scheme but with cell number per leaf area instead of A_{chl}/A was proposed for boreal plants of different life forms (Ivanova, 2012) and ecological groups (Ivanova, 2014) and for aquatic plants (Ronzhina et al., 2010). Our study in Central Asia as well as in European steppes (Ivanova et al., 2018) revealed that the chloroplast number per leaf area and A_{chl}/A rather than mesophyll cell number reflected plant response to arid climate. Clear differences between C_3 and C_4-plants with very low values of A_{chl}/A for the latter are evident from Fig. 6. As mentioned earlier by Longstreth et al. (1980), most C_4-species are generally characterized by low A_{mes}/A in comparison to C_3-species. Low values of integrated mesophyll parameters were also demonstrated for C_4-species in European steppes (Ivanova et al., 2018).

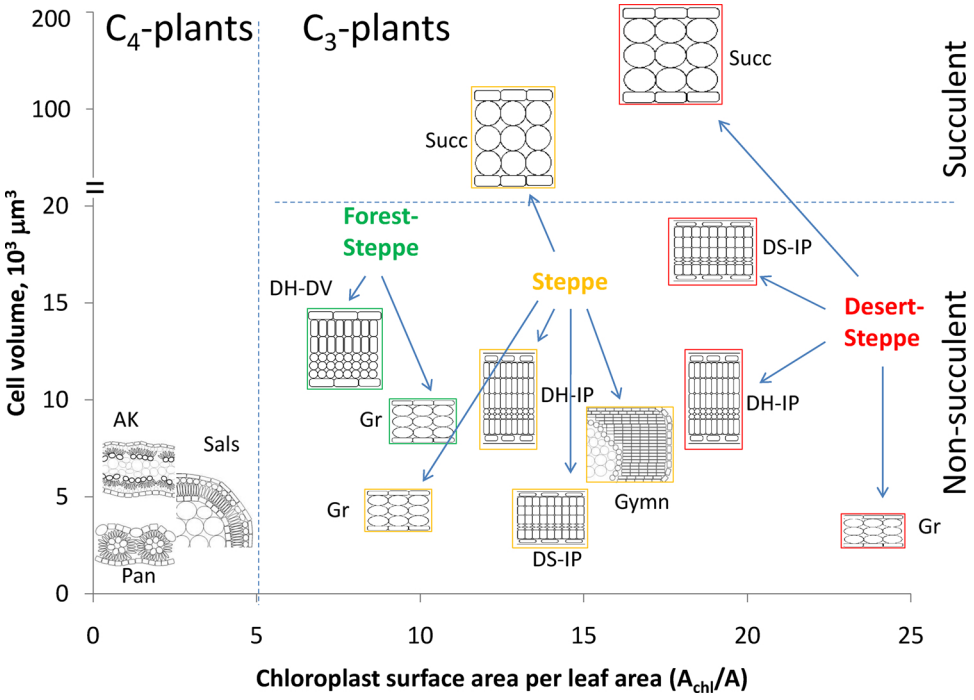

Fig. 6. General schema describing the functional diversity of semi-arid and arid vegetation. PFTs and zonal vegetation types are presented on a plane with two main traits of mesophyll structure as axes. C_4 – plants: AK – atriplecoid and kochioid types of kranz-anatomy, Pan – panicoid type, Sals – salsoloid type. C_3 – plants: DH-DV – dicotyledonous herbs with dorsiventral mesophyll, DH-IP – dicotyledonous herbs with isopalisade mesophyll, DS-IP – dicotyledonous shrubs, dwarf shrubs, semi-shrubs with isopalisade mesophyll, Gr – monocotyledonous herbs with graminoid type of mesophyll, Gymn – Gymnosperms *(Ephedra)*, Succ – herbs and shrubs with leaf succulence including species from *Alliaceae*.

The significance of differences between leaf structural types using *t*-test at *, $p \le 0.05$; **, $p \le 0.01$; ***, $p \le 0.001$. fs – forest-steppe, s – steppe, ds – desert-steppe. Cell volume: the differences are significant between Succ and all other groups, DH-DV-fs/DS-IP-s**, DH-DV-fs/Gr-s*, DH-DV-fs/Gr-ds*, Gr-fs/Gr-ds*, Gr-s/DH-IP-ds*, Gr-s/DS-IP-ds*, Gr-s/Gymn*, DH-IP-s/DS-IP-s*, DS-IP-s/Gymn*, DS-IP-s/DS-IP-ds***, DS-IP-s/DH-IP-ds***, DS-IP-ds/Gr-ds**. A_{chl}/A: Succ-s/Succ-ds*, DH-DV-fs/DH-IP-s*, DH-DV-fs/DH-IP-ds*, DH-DV-fs/DS-IP-s***, DH-DV-fs/DS-IP-ds***, DH-DV-fs/Gr-ds***, DH-DV-fs/Gymn**, Gr-fs/Gr-ds***, Gr-s/DH-IP-ds**, Gr-s/DS-IP-s*, Gr-s/DS-IP-ds*, Gr-s/Gymn*, DH-IP-s/DH-IP-ds**, DH-IP-s/Gr-ds***, DS-IP-ds/Gr-ds*.

The results presented here provide an evident explanation of how different PFTs within C_3-plants reach similar values of integrated parameters in the current climate. The cell-level mechanism is probably connected with biological peculiarities of species. For example, shrubs in comparison to herbs usually have deeper root systems that reach underground water supplies. Therefore, because of longer roots reaching the groundwater table, shrubs are usually more resistant to drought and, in our opinion, can use available water for maintaining cell turgor pressure. Only shrubs increase cell size and chloroplast number per cell with increasing aridity (Fig. 6). This mechanism is beneficial and is structurally less expensive than cell proliferation, involving increasing N_{cell}/A and N_{chl}/A. Herbs with a rather short root system are adapted to aridity by an increase in cell concentration per leaf area unit. Steppe grasses as well as steppe dicotyledonous herbs possess smaller cells than those in forest-steppe. In the latter case, the decrease in cell size is a probable mechanism contributing to WUE in the herbal growth form. We identified a considerable increase in cell number per leaf area for desert-steppe grasses as well as for dicotyledonous herbs in comparison with steppe and forest-steppe – a trait that definitely leads to enhanced A_{mes}/A and A_{chl}/A. Another type of leaf adaptation to arid climate is leaf succulence. C_3-succulents are characterized by large cell volume exceeding in several times all other PFTs. C_3-succulents raised their A_{mes}/A and A_{chl}/A by means of enlarging both cell size and cell number. Only two species among studied plants exhibit Crassulacean Acid Metabolism (CAM) –*Orostachys spinosa* and *Orostachys malacophylla* (Tsendeekhuu and Black, 2005). We found their characteristics very close to C_3-succulent species with high water content, leaf thickness and huge mesophyll cells (see in Results, not shown in figures). Here an exceptional structural type is *Ephedra*-type, representing the group of congeneric species of gymnosperms, in which aphyllous assimilating branchlets are characterized by high thickness (1.2–1.4 mm) and density (400–800 g m^{-2}), low proportion of photosynthetic tissue (45–60%) and low water content (45–55%). Nevertheless, the definite combination of cell size and number forms a large internal assimilation surface in *Ephedra* branchlets represented by the values of A_{mes}/A and A_{chl}/A, that are appropriate for adaptation to steppe vegetation environment. Indeed, the majority of *Ephedra*-species are characterized by botanists as montane-steppe (Grubov, 1982; Malyshev and Peshkova, 1984) (Table A.1 in Supplementary material).

Currently, there is a lack of data on the proportion of plants with different mesophyll types along geographical gradients (Gillison, 2013). In European steppes, a decrease in the proportion of species with DV mesophyll and an increase in isopalisade- and kranz-anatomy with aridity were shown (Ivanova et al., 2018). The same tendency to change in the occurrence of the mesophyll structures of C_3-plants along a humidity and light gradient was demonstrated for boreal plants in the Ural region, where in wet and shady habitats species with homogeneous mesophyll were predominant, in middle humid and moderately insolated places species with dorsiventral mesophyll, and finally in dry and well insolated sites species with isopalisade mesophyll (Ivanova, 2014). These works also demonstrate that the integrated mesophyll traits change along the moisture gradient. In the current study, which is more extensive, we also observed the change in the distribution of PFTs and zonal vegetation types along the aridity gradient that underpinned the shifts in site-mean values of mesophyll parameters inside C_3-plants.

The increase in proportion of C_4-plants with aridity has been shown for Mongolia (Pyankov et al., 2000). Among C_4-plants, the biochemical subtype is shown to be the most indicative trait response to environment. According to our findings, C_4-Salsoloid species have much thicker and denser leaves or aphyllous branchlets with higher A_{mes}/A and A_{chl}/A, compared to C_4-Atriplecoid and C_4-Kochioid types, and especially than C_4-Panicoid type (C_4-grasses). Indeed, arborescent species having a salsoloid type of Kranz anatomy and NADP-malic enzyme type were the most resistant to drought stress and severe environments in extreme arid Gobi deserts with less than 100 mm of annual precipitation, whereas C_4-grasses with NAD-ME and PEP-carboxykinase photosynthetic types were mainly annual and perennial herbs that occurred mostly in the steppe zone where they are often predominant in pasture ecosystems (Pyankov et al., 2000).

4.3. Why mesophyll traits are relevant indicators of response to climate aridity

Our results imply an important role of mesophyll structure and primarily the value of summarized cell surface area (A_{mes}/A) and chloroplast surface area (A_{chl}/A) per leaf area unit in plant adaptation to aridity. These parameters are not only anatomical features but also characterize exchange surfaces for CO_2 when it passes from the intercellular spaces to the chloroplast stroma. Therefore these parameters contribute substantially to mesophyll conductance (g_m). The change of g_m can influence A_{max} independently of stomatal conductance (g_s) (Flexas et al., 2013; Bahar et al., 2018). It was shown earlier that dry-site species tend to have higher A_{max} at a given stomatal conductance (Wright et al., 2001, 2003; Reich et al., 2003), which can cause a decrease of intercellular CO_2 concentration (C_i) (Wright et al., 2001; Prentice et al., 2014). High atmospheric vapour pressure deficit implies low C_i for plants in dry climates (Stewart et al., 1995; Prentice et al., 2014). Therefore, changes in mesophyll traits as the basic determinants of g_m should be important for plants for maintaining the vitally important level of A_{max} in arid climates.

According to the first Fick's law, there are two main mechanisms for regulation of CO_2 diffusion inside a leaf – a change in concentration gradient and a change in exchange surface area (Nobel, 1999; Tosens et al., 2012), which can be expressed by indices A_{mes}/A and A_{chl}/A. C_3- and C_4-plants differ in these mechanisms regulating the CO_2 flow rate. C_4-plants possess a special CO_2-concentrating mechanism (Black, 1971; Caldwell et al., 1977), which provides steeper concentration gradients. Indeed, the CO_2 diffusion conductance in mesophyll is higher in C_4-plants than that in C_3 ones (Longstreth et al., 1980; Kolbe and Cousins, 2018). Our results also showed that the rate of CO_2-transfer through both cell and chloroplast surface is higher in C_4-plants than in C_3-plants (Table 2). Furthermore, according to other sources (Su, 2010) photosynthesis rate for desert C_4-plants could be much higher than observed in the majority of our data. In the case of *Haloxylon ammodendron* and *Calligonum mongolicum*, A_{max} was shown 36.1 μmol CO_2 m^{-2} s^{-1} and 47.1 μmol CO_2 m^{-2} s^{-1} accordingly. These data for A_{max} could mean that species of C_4-Salsoloid type could have even much higher values of TR_{mes} – up to 2.9 μmol CO_2 m^{-2} s^{-1}, and higher TR_{chl} – up to 15–17 μmol CO_2 m^{-2} s^{-1}, and higher photosynthetic activity of chloroplasts in bundle sheath cells (A_{max}/chl) up to $2.2 \cdot 10^{-10}$ μmol CO_2 s^{-1}. The high rate of CO_2-transfer through the mesophyll and chloroplast surface in C_4-plants allows them to have lower area of the intra-leaf exchange surfaces and to invest less resources for the support the structural complexity of the mesophyll; as a result, C_4-plants even in the hyper-arid climate of Gobi desert are able to keep the same low values A_{mes}/A and A_{chl}/A as indicated in C_3-plants in a humid climate (Pyankov et al., 1998; Ivanova, 2014, Ivanova et al., 2018).

C_3-plants having a C_3-carboxylation reaction are constrained in regulation of CO_2 flow rate mainly by changes in the size of exchange surfaces. In the leaves of C_3-plants CO_2 diffusion but not the biochemical capacity of CO_2-assimilation is predominantly affected by drought stress (Flexas et al., 2004). In C_3 plants, mesophyll conductance has been linked to WUE (Barbour et al., 2010) and has been proposed as a way to enhance WUE whilst avoiding the reductions in photosynthesis typically seen when reducing g_s (Flexas et al., 2013). Indeed, we found a considerable increase of WUE in desert-steppe C_3-plants in comparison to steppe and forest-steppe (Table 2). The desert-steppe plants did not differ from the latter in LT or LMA, but had twice the A_{chl}/A. An increase in A_{chl}/A can furthermore provide the maximal degree of covering the cell surface by chloroplasts (k), as is shown in Fig. 5 by high values of parameter k for desert-steppe and desert C_3-plants. Only a few C_3-plants were found in hyper-arid deserts, where C_4-plants were

predominant. Desert C_3-plants were characterized by the same high values of integrated mesophyll traits as desert-steppe ones, and besides they had other values of pigment forms ratio – decreased chlorophyll *a*/*b* and increased chlorophylls/carotenoids. Similar trends in the pigments forms' ratio was found in steppes in the South Ural as a possible adaptation to aridity (Ivanov et al., 2013) – a factor that underpins changes in the functioning of photosynthetic units in chloroplasts under severe arid stress. The changes in carboxylation such as Rubisco activity (Wright et al., 2003; Prentice et al., 2014) or other biochemical mechanisms facilitating the diffusion of CO_2 (Evans et al., 2009) could be also expected in a few non-succulent C_3-species capable of surviving in a severe arid climate when they reach possible structural limits by an increase in mesophyll exchange surface. Our results clearly show that the increase of intra-leaf assimilation surface is one of the important mechanisms of adaptation of C_3-plants to arid climate and thereby such mesophyll parameters as N_{chl}/A, A_{mes}/A and A_{chl}/A are confirmed as good predictors of plant response throughout global aridity gradients. In C_4-plants having other mechanisms of adaptation to aridity concerning their biochemical peculiarities probably the biochemical subtype is predominant for adaptation to environment.

Thus, we found strong correlations of site-mean values of mesophyll traits with aridity along the large latitudinal gradient in Southern Siberia and Mongolia for C_3-plants. Leaf traits of C_3- and C_4-plants differed in response to aridity and showed opposite trends due to different mechanisms contributing to leaf mesophyll conductance. Patterns of C_3- and C_4-plant leaf types across semi-arid and arid regions in Central Asia have been generalized on a plane of mesophyll parameters. Cell size as well as chloroplast number per cell were clearly linked with plant functional type (PFT) whereas integrated mesophyll parameters were in greater degree related to vegetation types. N_{chl}/A and A_{chl}/A increased from forest-steppe and steppe to desert-steppe and desert C_3-plants. C_4-plants possess lower values of integrated mesophyll parameters compared to C_3-plants but higher rate of CO_2-transfer through the cell and chloroplast surfaces. We suppose that quantitative changes in leaf mesophyll structure allow different PFTs to maintain the photosynthetic performance under arid stress and to adapt to climate along a large latitudinal gradient.

Acknowledgements

The authors are deeply indebted Joint Russian-Mongolian Complex Biological Expedition RAS and MAS, who supported the field observation and data collection used in this study. The analytical part of this research has been partially supported by RFBR grant 17-29-05019, and partly by project №AAAA-A17-117072810011-1 of Russian Federal Budget. The research of O.A. Anenkhonov was carried out using the framework of project № AAAA-A17-117011810036-3 supported by the Russian Federal Budget.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.flora.2018.10.008.

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